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RECOMMENDED GUIDELINES FOR THE CONTROL AND ERADICATION OF MYCOPLASMA GALLISEPTICUM INFECTION IN CHICKENS AND TURKEYS

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INTRODUCTION

Despite progress in the control of Mycoplasma gallisepticum infection in chickens and turkeys, the disease resulting from this organism costs the poultry industry millions of dollars annually. These proposed guidelines for control and eradication of Mycoplasma infection in chickens and turkeys have been compiled to aid in unifying and intensifying efforts to combat the disease. The recommendations are the result of many meetings of scientists, industry representatives, and regulatory officials, and of the thinking of some of the best-known authorities on M. gallisepticum. These guidelines reflect the experience of scientists in State and Federal disease control laboratories and are based on their success in establishing and maintaining flocks free of M. gallisepticum infection.

This publication describes the characteristics and detection of the organism, tells how the disease affects chickens and turkeys, and tells what can be done about it.

The recommended procedures were developed from the guidelines adopted at many conferences and seminars based on research findings on the control and eradication of Mycoplasma infection, such as the Work Conference held May 28-30, 1962, in Washington, D.C.; the Study Group Conference on Mycoplasma gallisepticum infection held April 27-28, 1965, in Washington, D.C.; the Seminar on Avian Mycoplasma held January 1967, in Washington, D.C.; and the Workshop of the Western Poultry Disease Conference held March 20, 1968, in Davis, Calif. Representatives of the chicken (broilers and layers) and turkey industries, poultry disease researchers, and veterinary regulatory officials from various poultry producing States attended these meetings.

The recommendations are also based on experiences of poultrymen in implementing control measures.

These recommendations are an attempt to achieve a reasonable approach to the problem of M. gallisepticum infection and not an attempt to promulgate an official control program. It should be emphasized that the standards outlines in this report are minimal; more extensive programs may be needed in some areas.

ECONOMIC SIGNIFICANCE

In the United States in 1967, condemnation of birds having airsacculitis at time of slaughter or Mycoplasma caused the loss of $24.5 million. The owner's expense for treating the disease, the morbidity, and the cost borne by the industry for testing are estimated at $70.1 million. Federal and State expense

for conducting programs is $398,161. This totals $95 million for the cost of the disease in 1967.

Projecting the costs based on the rates from 1961 to 1967, the losses will be $123.5 million in 1970, if Mycoplasma is permitted to follow its present course.

GOALS

The overall goal of the program proposed herein is to eradicate Mycoplasma gallisepticum infection from our turkey and chicken flocks in as short a time as possible. Steps considered essential to prevent reinfection of flocks with M. gallisepticum are also included.

THE AGENT

Mycoplasma gallisepticum, which causes chronic respiratory disease in chickens and infectious sinusitis in turkeys, is a bacterialike agent composed of a three-layered membrane, without bacterial wall substances. This organism is pleomorphic and has fastidious growth requirements. Serum or serum substances are necessary for its growth. Very small characteristic colonies that grow into the agar and that have a raised center are seen on a solid medium.

Distinguishing characteristics of three Mycoplasma are:

	M. gallisepticum	M. synoviae	M. meleagridis
Ferments sugar	+	+	-
Hemagglutinates	+	+	-
Hemadsorbs	+	?	-
Requires Diphosphopyridine-nucleotide (DPN)	-	+	-
Infects chickens	+	+	-
Infects turkeys	+	+	+
Grows on direct agar	+	-	-
Requires CO_2	-	+	-

EPIZOOTIOLOGY AND TRANSMISSION

In the control of Mycoplasma gallisepticum, egg transmission of the agent is a major factor. Bird-to-bird infection is another important factor. The contact may be either living or dead birds. M. gallisepticum can infect pheasants, partridge, and quail. One case is documented of its occurrence in the wild pheasant. All other reports are of infection in inoculated birds.

Circumstantial evidence indicates that M. gallisepticum may be transmitted by man moving from pen to pen. This mode of transmission is considered particularly important in the occurrence of breaks in mature birds. Under usual environmental conditions, the organism cannot be transmitted from inanimate objects. The survival time of M. gallisepticum on such objects is less than 24 hours. The spread of M. gallisepticum is influenced by other respiratory disease agents and by stress factors. Some means of transmission are still undetermined.

In infections caused by the inoculation of a very small number of organisms, positive serology and histopathology may be seen in the absence of clinical disease. Under commercial conditions where stress is minimal, titers may occur with the absence of symptoms occasionally.

In the turkey, infection alone with M. gallisepticum may cause major disease. Frank disease in laying chickens is usually confined to the upper respiratory tract, which causes essentially no economic loss. Meat-type chickens are intermediate between turkeys and laying chickens in their response to the pure infection.

M. gallisepticum has no human public health significance. There is no evidence of involvement in Mycoplasma of avian origin in diseases of man or other animals.

Under industry conditions, M. gallisepticum causes an important disease problem because it is both a basic infection and a complicating factor in poultry respiratory diseases. Experimental proof is lacking of the economic significance of M. gallisepticum in commercial laying flocks. Circumstantial evidence, however, indicates that performance is improved in laying hens free of this agent.

The following factors are those that appear to be improved in birds free of M. gallisepticum:

- Egg production.
- Hatchability.
- Quality of chicks.
- Performance, weight gain, feed conversion, and livability.
- Cost of medication.
- Evidence of airsacculitis.
- Respiratory diseases complex in older birds.
- Joint involvements.
- Incidence of encephalitis.

DIAGNOSIS AND IDENTIFICATION

The diagnosis of Mycoplasma gallisepticum infection is established by the flock history, the pathology, and by serology.

The major symptom of M. gallisepticum in chickens and turkeys is an inflammation of the respiratory tract, particularly the anterior airsacs and lungs. The disease may be so severe as to cause inflammation and formation

of caseous material in the thoracic cavity and may even extend into the wing and leg bones. Frequently there is difficult breathing (dyspnea), loss of appetite, and loss of weight.

Serologic means of diagnosis are:

- Plate-agglutination tests and tube-agglutination tests.
- Hemagglutination inhibition (HI) tests.
- Other tests.

Cultural methods for identification of M. gallisepticum are:

- Growth on artificial media.
- Embryo inoculation using clean eggs free of all Mycoplasma.
- Airsac inoculation of test birds (there should be a change from negative to positive serology).
- Tissue culture procedures.

M. gallisepticum may be identified by:

- Preparing an antigen from the isolate and performing an agglutination test by using this antigen with specific positive and negative serum.
- Using fluorescent antibody techniques on colonies of the organism.
- Testing growth inhibition by using specific antiserum.
- Conducting hemagglutination and hemagglutination inhibition tests with the cultures.
- Preparing agar gel precipitin tests.

Under practical field conditions, serologic methods are the most reliable for detecting M. gallisepticum infection. However, serologic methods are not 100 percent accurate. Serologic tests will detect infection in a population, but not necessarily in all infected individuals.

PROCEDURES FOR CONTROL

Controls must be applied on a flock basis. The primary effort is against egg transmission of the agent.

Egg Dipping

A basic breeding flock can be converted from Mycoplasma gallisepticum-positive status to a negative status in one generation by using the egg dipping method. The selection of the time of saving hatchery eggs is important in the infected flock. There should not have been an upward change in serological titer on two monthly tests of the flock. There should not be any active air-sacculitis found on autopsy of cull birds from the flock. Before selection of eggs, at least 100 days should lapse after active infection. The eggs should show good hatchability and the chicks should be of good quality. Small group rearing should be practiced if possible. Birds should be tested at 10-weeks of age and again during the pullorum testing period at 20 to 24 weeks.

Dipping chicken eggs in tylosin tartrate for the control of M. gallisepticum.--The primary objective of this process is to absorb a sufficient amount of antibiotic through the porous egg shell to destroy any Mycoplasma organisms contained in the egg.

Two methods have been used to accomplish this objective: (1) the temperature-differential method to create the vacuum inside the egg and (2) a vacuum pump method to establish the vacuum.

The temperature-differential method is the most common.[1] Only clean eggs should be used. Eggs may be prewashed if desired. The eggs are trayed and incubated in the usual manner at 37° C. for the time necessary to warm the eggs until the center of the eggs to be dipped have reached temperature of 37°.

To decontaminate the egg surface, the eggs should be fumigated with formulin and potassium manganate (0.6 g. $KMnO_4$, 1.2 g. formulin/ft.^{3}) at the farm before they reach the hatchery. If the eggs were not previously fumigated, fumigation should be done during the warmup period in the incubator.

The eggs are then withdrawn from the incubator and emerged in the cold antibiotic solution (4° C.), which contains tylosin tartrate at the level of 1,500 parts per million for 15 minutes. Organic iodine solution, 0.45 percent (wescodyne, iosan, or comparable organic iodine) is added to the dip solution to prevent microbial contamination. The temperature of the dipping solution is best maintained by mechanical refrigeration.

The eggs are withdrawn from the solution and allowed to dry at room temperature. When all the eggs have been dipped, they are then returned to the incubator.

Extreme care must be taken to prevent microbial contamination. The tylosin tartrate is very stable in this solution if it is kept cool. When egg dipping is practiced on a large scale, 15,000 eggs may be dipped in a 100-gallon solution. Ice may be added to the solution if necessary to maintain the proper temperature. It is important that the solution be maintained at 4° C. (40° F.) or below, at all times. Eggs may be reset in the incubator when dry or stored at proper handling temperature for up to 2 days without any loss of hatchability.

Embryo Inoculation, Chick Inoculation, and Test and Slaughter

Embryo inoculation with antibiotics is a relatively more expensive method than egg dipping. It is harder on chicken survival and allows for more manipulation error. Chick inoculation may also be used, but 100 percent effectiveness has never been demonstrated. The test and slaughter method on a group or flock basis is used successfully in the turkey industry. It may be too expensive and impractical for the chicken industry.

Medication

The medication of infected birds helps to control the manifestation of disease but does not eliminate egg transmission and will not sterilize the bird. It is not a practical method of eradication. Medication is most effective by inoculation, less effective by addition to the water, and least effective in the feed.

Fumigation

Fumigation of the egg has no effect on the transmission of M. gallisepticum.

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[1] The vacuum-pump method is described in a monograph by A. C. Voeten of the Netherlands entitled, "Control of Chronic Respiratory Disease in Chickens by Egg-Dipping in a Solution of Tylosin Tartrate."

Sanitation

Good sanitation practices should always be followed. Quaternary ammonium compounds are effective against M. gallisepticum.

Vaccination

Effective, practical vaccines against M. gallisepticum have not been produced. Controlled infection has not proved to be satisfactory in the prevention of egg transmission. In some cases, controlled exposure resulted in egg transmission, and the procedure cannot be recommended if eradication of M. gallisepticum is the ultimate goal.

ERADICATION PROGRAM FOR THE CHICKEN INDUSTRY

Testing

Primary Breeder Level

Primary breeder flocks, that is, those that contribute to the continuation of foundation lines must be 100 percent tested and found negative at the time the pullorum-fowl typhoid test is conducted (24 weeks). This includes all additions to the flock, until there have been two generations with no evidence of Mycoplasma gallisepticum. These tests must have been made by the State-approved laboratory using materials and procedures approved by the U.S. Department of Agriculture and the State Livestock Regulatory Authority in the State in which the farm is located.

Multiplier or Commercial Flocks

Birds, chicks, or eggs that come from primary breeders, which have been 100 percent tested for two generations without reactors, may be tested at a rate that will assure 95-percent confidence of disclosing 1-percent infection rate as follows:

Birds in flock	Birds to be tested	Birds in flock	Birds to be tested
Number	Number	Number	Number
100	95	1,000	258
200	155	2,000	277
300	189	3,000	284
400	210	4,000	287
500	224	6,000	291
600	235	8,000	293
700	243	10,000	294
800	249	20,000	296
900	254	50,000	297

Birds, chicks, or eggs from other than primary breeders that have been negative for two generations must be 100-percent tested at pullorum-fowl typhoid testing time.

Screening of breeders at all levels will be either by the tube- or plate-agglutination method. The hemagglutination inhibition test is more sensitive and will be used to confirm positive or suspicious results. When suspicious reactions occur in flocks considered to be M. gallisepticum free, testing for M. synoviae will aid in avoiding false findings of M. gallisepticum infection.

When M. gallisepticum organisms are isolated, the flock will be deemed infected.

Testing Materials and Procedures

Criteria for testing materials and procedures required to determine the status of flocks are spelled out to assure that:

- Only flocks that have met test requirements are determined to be Mycoplasma gallisepticum free.
- Only officially approved testing materials will be used in testing for M. gallisepticum infection. The test will be conducted by an official laboratory in accordance with standards outlined. Blood samples shall be drawn by an authorized agent of the Animal Health Division, ARS, USDA, or of the State Livestock Regulatory Authority. Tests will be conducted by an authorized laboratory using either the serum plate-agglutination test, the tube-agglutination test, the hemagglutination inhibition (HI) test, or a combination of two or more of these tests. An antigen approved by the U.S. Department of Agriculture and the State Livestock Regulatory Authority shall be used in conducting these tests and shall be performed in accordance with the recommendations of the manufacturer. Plate- or tube-agglutination titers from 1 to 10 or above are suspicious and should be checked further. A titer of 1 to 80 with the HI test is considered positive, and a titer from 1 to 40 is considered suspicious when using two hemagglutination units. Reactors should be necropsied and isolation of M. gallisepticum attempted. The inoculation of isolates into the air sac of test birds is used to validate infection. Laboratories should be supplied with standardized, lyophilized serum in order that the antigens used may be tested for uniformity of sensitivity and specificity.

Maintenance of Negative Flocks

In maintaining negative M. gallisepticum flocks, there are these important factors:

- The flock must be clean to start with.
- The operation must be on an all-in, all-out basis.
- The house must be cleaned and disinfected with an approved disinfectant before introduction of baby chicks.
- Doors should be locked at all times.
- Visitors, servicemen, feedmen, salesman, or neighbors should not be allowed in the house at any time.
- Caretaker should avoid visiting premises where other poultry are kept or poultry products are processed.

Recommended Management and Sanitary Practices for Breeder Flocks

Strict adherence to sanitary requirements for the housing and handling of flocks is essential for establishing and maintaining M. gallisepticum-free production flocks (both broiler and table eggs).

The sanitary safeguards and management practices given below should be followed to minimize the possibility of exposure to new infection or reduce the chances of spread within a flock.

Selecting and Handling of Chicks

Baby chicks must be delivered in new or disinfected shipping equipment, directly from the hatchery, by attendants wearing sanitized clothing and disinfected shoes.

Chicks from different flocks should not be mixed.

Vaccination protocols for Newcastle disease, infectious bronchitis, and such should be tailored to meet the requirements of the premise or area in which the flock is located.

Buildings and Facilities

Poultry houses should have floors that can be readily cleaned and disinfected. Dirt floors should not be used.

For protection against wild birds, poultry house should have less than 1-inch mesh screens.

At the entry room next to the door, all poultry houses should have a stiff brush and a pan containing an approved disinfectant.

The disinfectant solution should be changed daily. The caretaker should dip his footwear into the solution upon entering and leaving the house.

No domestic animal, including dogs, or cats, should be permitted to enter the poultry house.

The farm should have a disposal pit or incinerator for dead birds.

Members of the caretaker's household working or providing service where poultry are kept or poultry products are processed should avoid contact with M. gallisepticum free flocks.

Because poultry, meat, and eggs purchased for household use may carry M. gallisepticum infection, they should be kept away from flocks.

Tractors, dump trucks, or other equipment used for other purposes should be thoroughly cleaned and disinfected before being returned to the premise.

Desirable Practices Whenever Possible

The farm should have bulk feed with outside filler pipes. No feed delivered should have been handled in used bags.

Medications, such as coccidiostats, that are currently used should be premixed in the feed before delivery.

Birds of different ages should not be kept on the premises at the same time unless they are housed in different buildings spaced at least 1,000 feet apart.

All poultry houses should be located no closer than 100 feet from public highways and not closer than 1,000 feet to poultry houses on adjacent premises.

All houses should be located at least 1,000 feet from poultry litter piles and other vermin attracting debris.

The building should have an outside oil filling pipe when fuel oil is used.

Testing

Primary Breeder Level

To qualify for Mycoplasma gallisepticum-free flock rating at the breeder level, turkey flocks must be hatched from two generations (great grandparents and grandparents). These flocks must test M. gallisepticum negative at pullorum-fowl typhoid test time (4 months) at a rate that will give 95 percent assurance of disclosing 1-percent infection (see tabular data on page 6). Tests must be administered by an officially approved laboratory using materials and procedures approved by the U.S. Department of Agriculture and the State in which the flock is located (see section on testing materials and procedures for details).

Parent Level--Multiplier and Commercial

At the parent level, birds, poults, or eggs must be from clean flocks; and parent flocks must test negative at a rate that will give 95 percent assurance of disclosing 1-percent infection rate (see tabular data on page 6), at pullorum-typhoid testing time.

In tests at all levels (breeder stock, multiplier, and commercial), if the flocks look suspicious in the tube test or plate test, an hemagglutination inhibition (HI) test will be applied. If M. gallisepticum organism is isolated, the flock will be deemed infected and should not be used for breeding purposes.

Suspicious flock will also be cross-tested for M. synoviae infection to avoid a false finding of M. gallisepticum infection.

Testing Materials and Procedures

All tests shall be conducted with an antigen approved by the Animal Health Division of Agricultural Research Service, U.S. Department of Agriculture, and the State Livestock Regulatory Authority. Such tests shall be considered in determining the classification of a flock or hatchery.

If any reactors are found, they shall be presented to an official laboratory within 10 days from date of test for post mortem and bacteriological examination. The reactors shall be accompanied by a shipping permit on which is recorded the individual band numbers. The following criteria shall be used to determine if the flock can receive an official classification as M. gallisepticum tested:

- Active air sac lesions.
- Recovery of M. gallisepticum organisms.
- Supplemental serological tests.

Flocks, which contain birds that are serologically positive but show no active air sac lesions from which M. gallisepticum organisms are not recovered and HI tests are negative, shall be considered suspicious. A 100 random, or 10 percent sample test--whichever is higher--will be conducted

on all suspicious flocks before the time they come into production. A negative random sample will qualify the flock for M. gallisepticum-free rating. If any of the random samples test positive to the plate or tube test, the final determination of flock rating will be based on the same criteria as outlined above. Additional tests shall be conducted if deemed necessary.

Flock Inspections

Each breeder flock (primary and parent) will be inspected at least once during the production period by a representative of the State Livestock Regulatory Authority. The inspector shall consider:

- Sanitation of buildings and premise.
- Signs of disease in the flock.
- Segregation of flock from other poultry and animals.

Hatchery Inspections

Each hatchery will be subject to periodic inspections by a representative of the State Livestock Regulatory Authority. The inspector shall consider:

- Hatchery sanitation practices.
- Identification of all hatching eggs.
- Disposal of hatching waste.
- Cleaning, disinfecting, and fumigating as recommended by official of the State Livestock Regulatory Authority.

The owner and hatcheryman are responsible for reporting immediately any respiratory infection in a breeder flock to the State Livestock Regulatory Authority. A breeder flock found infected with M. gallisepticum will be eliminated and hatchery eggs removed from incubators.

Primary and Parent Breeders Having Breaks

Primary breeders that have undergone a M. gallisepticum break on any one of its farms must be negative to a test of 100 percent of the replacement breeders placed on that farm following depopulation. Periodic flock inspections will also be made. All breeders maintained on that farm must remain free of M. gallisepticum for at least 6 months before recommended partial tests may be used to qualify for free status. These flocks will be maintained under surveillance until marketed.

Farms on which parent breeder flocks have experienced M. gallisepticum breaks, the replacement breeder flocks must be 100 percent tested at pullorum-fowl typhoid testing time and found negative. Surveillance on these flocks will be maintained until breeders are marketed. If no further breaks occur, the recommended partial testing may be used for these flocks to qualify for M. gallisepticum-free status.

Recommended Management and Sanitary Practices

Breeder Flocks

Maintain breeder flocks on farms separate from grower flocks.

Avoid introducing new breeding stock unless the birds can be adequately segregated for 30 days, or until proved to be free of Mycoplasma organisms.

Prevent transmission from outside sources by indirect contact through contaminated equipment, footwear, clothing, and vehicles.

Provide adequate isolation for breeder flocks to avoid airborne transmission from other flocks.

Minimize contact of breeder flocks with game and free-flying birds.

Eliminate chickens and other fowl from breeder farm.

Keep the rodent population and other pests under control.

Initiate a vaccination program for other poultry diseases aimed at the needs of the farm and area.

Allow no visitors to turkey houses or range premises.

Clean and disinfect all equipment after each use.

Maintain a footbath with an approved disinfectant at entrance to each pen.

Clean and disinfect confinement house or pole barn between blocks.

Use clean and well-drained areas for range.

Use clean dry litter free of mold.

Keep accurate records of death losses.

Dispose of all dead birds properly and promptly.

Seek services of a veterinary diagnostician, if abnormal losses or signs of disease occur.

Hatcheries (Multiplier Level)

Permit inspection of buildings, equipment, and poultry products contained therein at any reasonable time by agents of the Animal Health Division, ARS, USDA, or official of the State Livestock Regulatory Authority.

Maintain identity of hatching eggs as to flock and place of origin.

Keep hatchery and incubator room well isolated from battery room.

Practice recommended procedures for fumigation of incubators and hatchers.

Use only new egg cases or used egg cases that are clean and that have been fumigated between each use.

Maintain available and adequate records to show origin of all hatching eggs and destination of poults sold for the current year and one year previous.

Commercial Flocks

Maintain poultry buildings and premises in a sanitary condition.

Permit inspection of flock and premises at any reasonable time by agents of USDA's Animal Health Division or official of the State Livestock Regulatory Authority; submit flock for collection of additional blood samples, if deemed necessary.

Attempt to eliminate animal disease carriers, such as rats and mice. Discourage the presence of free-flying birds.

Avoid raising other poultry and farm animals on premises unless well segregated.

Report immediately to a disease control official when any respiratory symptoms appear in the flock.

Refrain from using any drug that will mask the results of serological tests or bacteriological recovery.

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